

STIC-ILL

370228
10/15

From: Hunt, Jennifer
Sent: Sunday, November 04, 2001 12:29 PM
To: STIC-ILL
Subject: References for 09/218,481

Please send me the following references ASAP:

JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM, (1998 Aug) 18 (8) 887-95
Adv. Exp. Med. Biol. (1998), 454(Oxygen Transport to Tissue XX), 311-317
BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (1998 Sep 18) 60 (1) 89-97
JOURNAL OF APPLIED PHYSIOLOGY, (1998 Jul) 85 (1) 53-7
STROKE, (1997 Oct) 28 (10) 2039-44
NEUROSURGERY, (JUN 1997) Vol. 40, No. 6, pp. 1269-1277
NEUROSURGERY, (1997 May) 40 (5) 1016-26
JOURNAL OF CLINICAL INVESTIGATION, (1996 Sep 15) 98 (6) 1400-8
JOURNAL OF NEUROSURGERY, (1996 Dec) 85 (6) 1095-101
Proc Annu Meet Am Assoc Cancer Res, (1996). Vol. 37, pp. A399
Oncology Reports, (1995) 2/6 (1147-1149)
NEUROSURGERY, (1994 Sep) 35 (3) 439-48
YALE JOURNAL OF BIOLOGY AND MEDICINE, (1993 Jul-Aug) 66 (4) 277-314
MOLECULAR BIOLOGY OF THE CELL, (1993 Jan) 4 (1) 121-33
Endothelial Cell Dysfunct. (1992), 477-503
J CELL BIOL. (1990) 111 (5 PART 2), 227A.

Thanks,

Jennifer Hunt
Patent Examiner, Art Unit 1642
CM1-8D06 (mailbox 8E12)
(703)308-7548

Cost - Yr. No

CAS 116

RUSH FAX

THE BRITISH LIBRARY D.S.C.

FAX TRANSMISSION IN RESPONSE TO

A COPYRIGHT FEE PAID REQUEST

COPYRIGHT: OUR LICENCE EFFECTIVELY RESTRICTS FAX TO PAPER TO PAPER DELIVERY. VIEWING THIS DOCUMENT ON A SCREEN OR CONTINUING TO STORE IT ELECTRONICALLY AFTER THE RECEIPT OF A SATISFACTORY PAPER COPY, IS NOT PERMITTED.



This document has been supplied by
The British Library Document Supply Centre,
on behalf of
Chemical Abstracts Service.
Warning: Perfect copying of this document,
(including storage in any medium by microfilm means),
other than that allowed under the copyright law, is not
permitted without the permission of the copyright
owner or an authorized licensing body.



CAS Document Detective Service
2540 Olentangy River Road
P.O. Box 3012
Columbus, OH 43210-0012

Expression of vascular endothelial growth factor in the cyst fluid of human cerebral gliomas

SHAHRIAR KOOCHAKPOUR¹, PETER BULLOCK¹, ANDREW F. DEAN¹,
GEOFFREY J. PILKINGTON¹ and ABDERRAHIM MERZAK^{1,2}

¹Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK;

²Département de Biologie, Faculté des Sciences, Université Moulay Ismail, Meknès, Morocco

Received July 4, 1995; Accepted August 8, 1995

Abstract. Vascular endothelial growth factor (VEGF) or vascular permeability factor (VPF) has been shown to play a key role in angiogenesis in several solid tumours including human brain neoplasms. Its expression has also been found to be correlated to malignancy in the major class of these tumours, gliomas. Moreover, it has been suggested that cyst fluids (CFs) associated with human gliomas may contain a permeability factor responsible for the formation of brain edema and disruption of the blood-brain barrier generally observed in these tumours. We demonstrate that VEGF is present in low and high grade gliomas of seven patients. We also show that VEGF concentration increases with increasing malignancy of the tumours. Although further cases should be investigated, these results suggest that the amount of CF-VEGF may be of value in the diagnosis of human gliomas.

Introduction

A major hallmark of gliomas, the major form of human intrinsic brain tumours, is their striking neovascularization (1). Angiogenesis is essential for proliferation of the primary tumour as well as the secondary foci arising as a result of tumour spread. One factor which has been shown to play a central role in tumour angiogenesis, in general, and in glioma neovascularization, in particular, is the vascular endothelial growth factor, VEGF (2). Indeed, the VEGF gene was originally cloned from human glioblastoma cells as an angiogenic factor homologous to the platelet derived growth factor, PDGF (3). It was also colocalised with another well known angiogenic agent, basic fibroblast growth factor (bFGF), in neoplastic glial cells surrounding blood vessels

(2,4,5). VEGF is produced by human glioma cells to act in a paracrine fashion on receptors expressed almost exclusively on endothelial cells of neoformed blood vessels (2,6,7). VEGF specific antibodies inhibited both tumour angiogenesis and tumour growth demonstrating the critical role of this factor in tumour progression *in vivo* (8). This is substantiated by a recent report showing that a dominant-negative mutant of the receptor flk-1 prevented the growth of glioblastoma cells *in vivo* without affecting their growth *in vitro* (9).

VEGF was initially described as a vascular permeability factor (VPF) owing to its capacity to increase capillary vascular permeability after intradermal injection into guinea pigs (10). This experiment suggested that VPF may be responsible for the vasogenic brain edema that is a characteristic of human gliomas and metastatic intracerebral tumours (10). Indeed, the marked neovascularisation of human malignant gliomas is generally associated with cerebral edema, vein thrombosis, and tumour necrosis (1). Occasionally, human gliomas are also associated with formation of a cyst fluid (CF) (1). Although, the composition of CF is not completely known, few reports demonstrated the presence of hormones, cytokines, and growth factors in CFs of human brain tumours. Prisell *et al.*, (11) demonstrated the presence of the growth-promoting hormones, somatomedins, in tumour cyst fluid taken from patients with glioma at elevated levels compared with normal serum levels. Active forms of the strong immunosuppressor transforming growth factor beta 2 (TGF- β 2) were also detected in tumour cyst fluid from glioblastoma patients (12). The cyst fluid of human gliomas has also been found to contain interleukin 8 (IL-8) (13), insulin-like growth factor (IGF)-I, -II and IGF-binding proteins (14), and monocyte chemoattractant protein-1 (MCP-1) (15). Interestingly, Whittle *et al.*, (16) reported, in a feline infusion model of brain edema, that human glioma cyst fluid infusates increased local white matter water content and caused marked extracellular edema. This was associated with significant endothelial and astrocytic swelling, focal endothelial necrosis, and basement membrane disruption. These results suggested the existence of glioma derived permeability factors in CFs. In this study, we set out to investigate this hypothesis.

Correspondence to: Dr A. Merzak, Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK

Key words: endothelial growth factor, cyst fluid, cerebral gliomas

Materials and methods

Materials. Vascular endothelial growth factor and antibodies for ELISA assays were from R & D Systems Europe Ltd. (Abingdon, UK). All other chemicals for ELISA assays were obtained from Sigma Chemical (Dorset, UK). 96-well plates were from Marathon LS (London, UK).

TCF-sampling. Human CF samples were obtained by stereotactic aspiration or aspiration through an indwelling catheter at the time of craniotomy for therapeutic and diagnostic purposes. All samples were centrifuged to remove coagulated material, and the supernatants were frozen at -70°C until use.

ELISA assay. The 96-well plates were coated with 50 µl of samples per well for 48 hours at 4°C. The wells were washed 4 times with 300 µl/well of washing buffer (0.123 M NaCl, 0.01 M Na₂HPO₄, 0.05% Tween-20), blocked with 300 µl of 5% BSA in PBS and left at 4°C for 48 h. The plates were washed as above and 100 µl/well of mouse anti-hVEGF monoclonal antibody at a dilution of 1:1000 in PBS was added. Samples were incubated for 4 h at 4°C, then washed and 100 µl/well of biotinylated anti-mouse IgG at 1:1000 in PBS was applied for 1 h at room temperature. After 30 minutes incubation, the plates were washed again and 100 µl of avidin/biotin-horseradish peroxidase was added to each well for 30 minutes at room temperature. The plates were then washed five times and incubated with 100 µl/well of TMB (0.1 mg/ml) and H₂O₂ (1/1000) for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µl/well of 10% H₂SO₄. The amount of VEGF was quantitatively determined by the absorbance measured (at 570 nm) with an ELISA-plate reader. Concentrations of VEGF in test samples were determined by comparing their specific absorbance with those obtained for the standards plotted on a standard curve. Each assay was set in twelve replicates and repeated at least three times.

Results and Discussion

This study was carried out on samples taken from seven patients with cerebral gliomas. The results are summarized in Table I. Two of these patients, 35- and 30-year old, were diagnosed with grade II astrocytomas at the temporal lobe. VEGF was found to be present at 0.051 and 0.068 µg/ml in the CF of these two patients, respectively. Three patients; 43-year old, 37-year old, and 63-year old were diagnosed with grade III astrocytomas at the temporal lobe, the parietal lobe, and temporal lobe, respectively. The cyst fluid of these patients contained 0.098, 0.145, and 0.260 µg/ml of VEGF. It is interesting to note that the 37-year old patient was previously (1987) diagnosed with a grade II astrocytoma and underwent both radiotherapy and chemotherapy before recurrence with a grade III astrocytoma. The amount of VEGF in the CF of this patient was two times more than in grade II astrocytomas. The CFs of two patients (64- and 56-year old), diagnosed with glioblastoma multiforme at the temporal lobe and the parietal lobe, were found to contain 10 to 18 times more VEGF than the grade II astrocytomas, and

Table I. Presence of VEGF in cyst fluid of human gliomas.

Patients	Grade	VEGF (µg/ml)
1	II	0.051
2	II	0.068
3	III	0.098
4	III	0.145
5	III	0.260
6	Glioblastoma multiforme	0.680
7	Glioblastoma multiforme	0.960

2.6 to 10 times more than grade III astrocytomas. Although a more significant number of human gliomas has to be investigated, the data presented show that VEGF is indeed expressed in CF and may, therefore, explain the CF-induced edema and blood-brain barrier disruption observed in animal models (16). The results presented also suggest that the amount of CF-VEGF is correlated to the grade of malignancy; VEGF amounts being increased with increasing malignancy. This result is in agreement with the pattern of VEGF expression in human gliomas. Indeed, Plate *et al.* (2) demonstrated that VEGF is induced in astrocytoma cells but is dramatically upregulated in glioblastoma cells. In addition, they found that the high-affinity tyrosine kinase receptor for VEGF, flt-1, is also upregulated in tumour endothelial cells *in vivo*. The same and other authors recently reported (6,7) that both VEGF receptors, flk-1 and flt-1, are co-expressed in vascular cells in glioblastoma but not in low-grade glioma and that VEGF is present in significant amounts only in the vasculature of glioblastomas. Furthermore, a similar co-expression pattern of VEGF and its receptors has been reproduced in an animal model of human gliomas (17). The elevated production of VEGF in malignant gliomas versus low grade gliomas has been suggested to be due to the activation of epidermal growth factor receptor (EGF-r) in these tumours (18). We have also recently found that VEGF release is stimulated by growth factors and gangliosides, more prominently in malignant glioma cell lines (Koochekpour *et al.*, submitted). In conclusion, although more cases should be investigated, quantitation of VEGF in cyst fluid of human gliomas may prove to be a valuable tool in the diagnosis of human gliomas.

Acknowledgements

This work was supported by a grant from the Leverhulme Trust to GJP. SK was supported by a grant from the Iranian Ministry of Health, Education, and Medical treatment.

References

1. Russell DS and Rubinstein LJ: Tumours of central neuroepithelial origin. In: Pathology of Tumors of the Nervous System. (ed 5). Williams & Wilkens, Baltimore, MD pp83-289, 1989.

2. Plate KH, Breier G, Weich HA and Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. *Nature* 359: 845-848, 1992.
3. Conn G, Bayne ML, Soderman DD, Kwok PW, Sullivan KA, Palisi TM, Hope DA and Thomas KA: Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor. *Proc Natl Acad Sci USA* 87: 2628-2632, 1990.
4. Alvarez JA, Baird A, Tatum A, Daucher J, Chorsky R, Gonzalez AM and Stopa EG: Localization of basic fibroblast growth factor and vascular endothelial growth factor in human glial neoplasms. *Mod Pathol* 5: 303-307, 1992.
5. Shweiki D, Itin A, Soffer D and Keshet E: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 359: 843-845, 1992.
6. Plate KH, Breier G, Weich HA, Mennel HD and Risau W: Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible *in vivo* regulatory mechanisms. *Int J Cancer* 59: 520-529, 1994.
7. Hatva E, Kaipainen A, Mentula P, Jaaskelainen J, Paetau A, Haltia M and Alitalo K: Expression of endothelial cell-specific receptor tyrosine kinases and growth factors in human brain tumors. *Am J Pathol* 146: 368-378, 1995.
8. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS and Ferrara N: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 362: 841-844, 1993.
9. Millauer B, Shawver LK, Plate KH, Risau W and Ullrich A: Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature* 367: 576-579, 1994.
10. Bruce JN, Criscuolo GR, Merrill MJ, Moquin RR, Blacklock JB and Oldfield EH: Vascular permeability induced by protein product of malignant brain tumors: inhibition by dexamethasone. *J Neurosurg* 67: 880-884, 1987.
11. Prisell P, Persson L, Boethius J and Sara V: Somatomedins in tumour cyst fluid, cerebrospinal fluid, and tumour cytosol in patients with glial tumours. *Acta Neurochir Wien* 89: 48-52, 1987.
12. Bodmer S, Huber D, Heid I and Fontana A: Human glioblastoma cell derived transforming growth factor- β 2: evidence for secretion of both high and low molecular weight biologically active forms. *J Neuroimmunol* 34: 33-42, 1991.
13. Van-Meir E, Ceska M, Effenberger F, Walz A, Grouzmann E, Desbaillets I, Frei K, Fontana A and de-Tribolet N: Interleukin-8 is produced in neoplastic and infectious diseases of the human central nervous system. *Cancer Res* 52: 4297-4305, 1992.
14. Zumkeller W, Saaf M and Rahn T: Insulin-like growth factor (IGF)-I, -II and IGF-binding proteins in the cyst fluid of a patient with astrocytoma. *Childs Nerv Syst* 9: 100-103, 1993.
15. Kuratsu J, Yoshizato K, Yoshimura T, Leonard EJ, Takeshima H and Ushio Y: Quantitative study of monocyte chemoattractant protein-1 (MCP-1) in cerebrospinal fluid and cyst fluid from patients with malignant glioma. *J Natl Cancer Inst* 85: 1836-1839, 1993.
16. Whittle IR, Ironside JW, Piper IR and Miller JD: Neuropathological and neurophysiological effects of interstitial white matter autologous and non-autologous protein containing solutions: further evidence for a glioma derived permeability factor. *Acta Neurochir Wien* 120: 164-174, 1993.
17. Plate KH, Breier G, Millauer B, Ullrich A and Risau W: Up-regulation of vascular endothelial growth factor and its cognate receptors in a rat glioma model of tumor angiogenesis. *Cancer Res* 53: 5822-5827, 1993.
18. Goldman CK, Kim J, Wong WL, King V, Brock T and Gillespie GY: Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: a model of glioblastoma multiforme pathophysiology. *Mol Biol Cell* 4: 121-133, 1993.